A Comparative Analysis of Amphotericin B Lipid Complex and Liposomal Amphotericin B Kinetics of Lung Accumulation and Fungal Clearance in a Murine Model of Acute Invasive Pulmonary Aspergillosis

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Running title: Lipid AMB pharmacodynamics in aspergillosis

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Figures: 3

Abstract: 256

Word count: 3,658

Key words: Aspergillus, amphotericin B, lipid, liposomal, tissue concentration
ABSTRACT

The reformulation of amphotericin B (AMB) into a lipid complex (amphotericin B lipid complex, ABLC) or liposomal carrier (liposomal AMB; L-AMB) changes the rate and extent of drug distribution to the lung. The importance of pharmacokinetic differences among the various lipid AMB formulations in the treatment invasive pulmonary aspergillosis (IPA) remains unknown. We compared the kinetics of AMB lung accumulation and fungal clearance of ABLC and L-AMB treated mice with acute IPA. Balb-C mice were immunosuppressed with cyclophosphamide and cortisone before intranasal inoculation with 1.5 x 10^6 of A. fumigatus 293 conidia. ABLC or L-AMB was administered in daily IV doses (1, 5 or 10 mg/kg) starting 12 hours after infection and continued until D+5. At predetermined timepoints (0, 24, 72 and 120 hrs), mice were euthanized and lungs were harvested for determination of lung fungal burden (quantitative PCR) and total AMB lung tissue concentrations. Both ABLC and L-AMB were effective at reducing lung fungal burden at doses > 5 mg/kg/day. Clearance of A. fumigatus during the first 24 hours was associated with AMB tissue concentrations > 4 μg/g. At 5 mg/kg day, ABLC produced a more rapid fungal clearance than L-AMB, but at the end of therapy, fungal burden reduction was similar for both formulations and was not improved with higher dosages. These data suggest that ABLC delivers active AMB to the lung more rapidly than L-AMB, resulting in faster Aspergillus clearance in an experimental model of IPA. However, pharmacodynamic differences between the two formulations were less apparent when dosed at 10 mg/kg/day.
Despite the availability of new treatment options, lipid formulations of amphotericin B regimens continue to play a central role in the management of invasive pulmonary aspergillosis (IPA) due to their broad spectrum and low potential for cross-resistance with other antifungals (5). Currently, three lipid formulations are approved for the treatment of IPA in patients who have failed or are intolerant to other therapies: amphotericin B colloidal dispersion (ABCD), amphotericin B lipid complex (ABLC), and liposomal amphotericin B (L-AMB). All three of the amphotericin B (AMB) formulations differ in terms of their lipid composition and particle size, resulting in different pharmacokinetic characteristics when the drugs administered in vivo. For example, L-AMB consists of small unilamellar particles (60-70 nm) that avoid uptake by cells of the reticuloendothelial system (RES) (7, 8, 14, 25). Hence, intravenous administration of L-AMB results in sustained, high concentrations of encapsulated AMB in the serum with a somewhat delayed distribution of free drug to tissues. Conversely, intravenous administration of ABLC produces relatively low serum AMB concentrations due to the rapid RES cell uptake of the large lipid complex (1,600-11,000 nm) (1). Extensive RES cell uptake of ABLC is thought to account for the more rapid distribution of ABLC to certain organs, such as the lungs, compared to other formulations (18, 19). The clinical relevance of these pharmacokinetic differences between L-AMB and ABLC, however, remains unknown.

It is generally accepted that any delay in the initiation of antifungal therapy for IPA contributes to poorer clinical response (2, 11). In neutropenic hosts, unimpeded growth of Aspergillus hyphae in the lung results in hemorrhage and coagulative tissue necrosis with limited blood flow (27). Hence, it is unlikely that significant amounts of drug are delivered to infacted tissue containing the sequestered Aspergillus hyphae. Using a neutropenic rat model of IPA, Becker and colleagues found that delaying the start of L-AMB therapy (10 mg/kg/day) as little as 8
hours considerably increased animal mortality (6). Interestingly, L-AMB treatment was significantly more effective if a dose of conventional AMB-deoxycholate (1 mg/kg/day) was administered at the start of L-AMB treatment, suggesting that the availability of active AMB in the lung at early stages of infection was a critical factor in animal survival (6). The investigators did not test other lipid formulations such as ABLC, which is known to rapidly distribute to the lung after intravenous administration.

The goals of this current investigation were to: 1) compare the kinetics of AMB accumulation in lung tissue following intravenous treatment with ABLC or L-AMB, and 2) determine whether differences in the rate of tissue AMB accumulation between the two lipid formulations correlated with different rates of A. fumigatus clearance in an experimental model of acute IPA. (Presented at the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy 2006, San Francisco, CA, Abstract M-1689).

MATERIALS and METHODS

Reagents. Cortisone acetate and cyclophosphamide were obtained from Sigma Aldrich (St. Louis, MO). The commercial formulation of L-AMB (Ambisome®) was obtained from Amerisource-Bergen (Chesterbrook, PA). The human clinical formulation of ABLC (Abelcet ®) was generously provided by Enzon Pharmaceuticals (Bridgewater, NJ).

Animals. Eight week old female Balb-C mice (18-25 grams) (Charles River Laboratories) were used in all experiments. Mice were housed in sterilized filter top cages and had access to sterile food and water ad libitum. All mice were cared for in accordance with the highest standards for humane and ethical care as approved by the institutional animal care and use committee.
Inoculum preparation. Aspergillus fumigatus 293 (AF 293), the strain used for genome sequencing (21), was grown on potato dextrose agar for seven days prior to collection of conidia. Conidia were harvested from the slant with 0.1% Tween 20 in phosphate buffered saline (PBS) and passed through a syringe with sterile glass wool to remove hyphal fragments. The resulting suspension was then centrifuged for 5 minutes at 15,000 x g, the supernatant was discarded and the number of conidia were determined by hemocytometer counting. The final concentration was adjusted to 5 x 10^7 conidia. Harvested conidia were determined to be > 98% viable based on plating of a serially-diluted inoculum on Sabouraud dextrose agar. Susceptibility testing was performed using AMB epsilometer strips (AB Biodisk, Solna Sweden) and established methods for determining the mean fungicidal concentration (MFC) for filamentous fungi (24).

Immunosuppression and infection. Immune suppression was achieved by intraperitoneal (IP) injections of cyclophosphamide 75 mg/kg at 4 days and 1 day prior to infection. This regimen results in total polymorphonuclear neutrophil (PMN) depletion until 96 hours after infection (16). In addition, animals received a single 300 mg/kg intraperitoneal dose of cortisone acetate suspension prepared in PBS with 0.2% Tween-20 at 1 day prior to infection (31). Doxycycline HCl (Sigma) was added to the drinking water (0.5 mg/mL) as antibacterial prophylaxis. Additionally, doxycycline/drinking-water soaked mouse chow was placed in the corner of each cage and exchanged daily to reduce animal dehydration.

Prior to inoculation, animals were anesthetized with a single IP injection (200 µl) of ketamine 80 mg/kg + xylazine 10 mg/kg and placed on warming pads prior to intranasal inoculation with 1.5x10^6 A. fumigatus conidia. Animal inoculation was performed by slowly instilling a 30 µl droplet on both nares and the mice were allowed to inhale the inoculum in an upright position until normal breathing resumed (16). After inoculation animals were returned to the warming pad and...
observed until full recovery. This protocol results in reproducible infection of the lungs with untreated animals succumbing to the infection 96-120 hours after inoculation (16).

**Antifungal treatment and sample collection.** Groups of immunosuppressed mice (20 per treatment arm) received intravenous antifungal therapy with L-AMB or ABLC at a dose of 1, 5 or 10 mg/kg diluted in sterile 5% dextrose water and administered once daily by lateral tail vein injection. Control animals were administered D5W alone. In selected experiments, L-AMB and ABLC regimens were also administered as a single intravenous dose of either 5 or 10 mg/kg. All antifungal regimens were started 12 hours after inoculation and continued daily for 4 days. At serial timepoints after infection (T=0, 24, 72, and 120 hrs) five mice were euthanized by CO₂ narcosis and blood was immediately collected into a heparinized syringe by cardiac puncture. Blood was then transferred to a sterile capped tube and centrifuged (10,000 x g) for 10 minutes. Plasma supernatants were subsequently transferred to a beta glucan free cryovial and stored at -80°C until analysis. Lungs were then aseptically removed and stored at -80°C until analysis.

**Tissue fungal burden.** Pulmonary fungal burden was determined by real-time quantitative PCR by methods previously reported (9, 30). Briefly, DNA samples isolated from homogenized lungs were assayed in duplicate by use of an ABI PRISM 7000 sequence detection system (Applied Biosystems, Foster City, CA) using primers and a dual-labeled fluorescent hybridization probe specific for the *Aspergillus* 18S rRNA gene (9). The threshold cycle (Cₜ) of each sample was interpolated from a seven-point standard curve of Cₜ values prepared by spiking uninfected mouse lungs with known amounts of conidia (10¹ to 10⁷) from AF 293. An internal standard was amplified in separate reactions to correct for the percent difference in DNA recovery (30, 31). Results are reported as conidial equivalents (CE) of *A. fumigatus* DNA.
Amphotericin B tissue concentrations. Determination of total AMB tissue concentrations in infected lungs was performed by high-performance liquid chromatography using a modification of a previously published assays (15, 20, 26). Briefly, amphotericin B and a spiked internal standard (1-amino-4-nitro-naphthalene, ANNP) were isolated from tissue homogenates by acetonitrile (ACN) precipitation of proteins followed by centrifugation. A 50 μL aliquot of the extracted supernatent was injected through a C18 guard column into a Nova-Pak C18 column (3.9x150 mm, 4 μM). AMB and ANNP (internal standard) were eluted at a flow rate of 1 mL per minute with gradient program (ACN from 30 to 45% plus 2.5 mM EDTA from 70% to 55% in 8 minutes) and detected at 406 nm. The calibration curve was linear over a range of 0.25 to 10 μg/g in tissue. Mean inter- and intra-assay coefficients of variation over the range of the standard curve were < 10%. The lower limit of accurately detectable AMB in tissue was 0.25 μg/g.

Plasma beta glucan concentrations. Plasma beta-glucan (BG) concentrations in infected animals treated with L-AMB or ABLC was determined using a commercially-available assay according to the manufacturer’s instructions (Fungitell; Associates of Cape Cod). Plasma samples (5 μL) were pretreated for 10 min at 37°C with an alkaline reagent (20 μL; 0.125 M KOH/0.6 M KCl) to inactivate serine proteases as well as inhibitors in mouse plasma and to enhance the reactivity to activated factor G (28). After addition of the BG assay reagent, the microtiter plate was inserted into a pre-incubated microplate spectrophotometer (Powerwave X Select, Biotech Instruments, Winooski, VT) and a kinetic assay was run at absorbance 405 nm with 490 nm correction using the KC4 software (Biotech) on unknowns from infected animals or BG standards (15-250 pg/mL) provided by the manufacturer.
**Statistical analysis.** All data were expressed as means plus/minus standard errors of the means (SEMs) and compared by Mann-Whitney test or one-way analysis of variance with Tukey's post-test for multiple comparisons where appropriate. Differences were considered statistically significant when \( P \) values were < 0.05. Total AMB tissue concentrations associated with inhibition of *A. fumigatus* growth (stasis) or 1 log\(_{10}\) reduction in fungal burden at 24 hours were determined by fitting a four-parameter logistic model (Hill equation) using computer curve-fitting software (Prism 4, GraphPad Software, Inc, San Diego, CA, USA) to the experimental data. Goodness of fit was assessed by \( R^2 \) and the standard error of the EC\(_{50}\) value.

**RESULTS**

**Isolate susceptibility testing.** Susceptibility testing performed in triplicate of the test isolate AF 293, revealed an AMB MIC of 0.25 \( \mu \)g/mL and a MFC of 2 \( \mu \)g/mL. MIC and MFC results were consistent throughout the study and with previously published *in vitro* studies that utilized the same isolate (17).

**Kinetics of fungal burden reduction.** Plots of tissue fungal burden at 0, 24, 72, and 120 hrs versus total concentrations of AMB in lung tissue are presented in Figure 1. After inoculation, the mean baseline fungal burden in the lung was \( 5.86 \times 10^5 \) (range \( 1.31 \times 10^5 – 6.60 \times 10^6 \) *A. fumigatus* conidial equivalents (CE) DNA across all treatment groups. In control animals (D5W treated), fungal burden increased by 1 log\(_{10}\) by 72 hours, and was associated with onset of animal mortality at 72-120 hrs consistent with previous studies (9, 31).

Animals treated with either L-AMB or ABLC at 1 mg/kg/day exhibited increasing fungal burden until 72 hours similar to untreated (control) animals. Mortality was associated with fungal
burden in excess of approximately $5 \times 10^6$ CE occurring among all three groups between 72-120 hrs (Figure 1A). Therefore, experiments could not be completed until the 120 hr timepoint. AMB could only be detected in lung tissue for ABLC-treated animals at 24 hours (mean 4.2 µg/g ± 1.77), but was undetectable at later timepoints despite daily dosing and for all timepoints in L-AMB treated animals.

Animals treated with L-AMB or ABLC at 5 mg/kg/day exhibited a significant reduction in fungal burden vs. control animals at 72 hours ($P<0.05$). Clear differences in the pattern of fungal burden reduction were observed between the two lipid AMB formulations with ABLC producing immediate reductions in fungal burden by the first 24 hrs that was not seen with L-AMB until 72 hours (Figure 1B). Analysis of AMB tissue concentrations by HPLC confirmed higher concentrations of AMB in ABLC-treated animals at 24 hrs vs. L-AMB treated animals (34.2 µg/g vs. 2.09 µg/g, respectively; $P<0.05$). By 72 hours, the mean tissue fungal burden ($1.35 \times 10^5$ vs. $3.23 \times 10^5$ AF 293 CE) and AMB tissue concentrations (12.7 µg/g vs. 8.21 µg/g) were similar for ABLC and L-AMB treated animals dosed at 5 mg/kg/day (Figure 1B). The absolute difference in fungal burden between the two dosing regimens at 24 hours did not quite reach statistical significance ($P=0.06$).

ABLC or L-AMB treatment at 10 mg/kg/day achieved higher concentrations of AMB in the lungs at 24 hours (55.4 µg/g vs. 15.0 µg/g, respectively; $P<0.05$) and faster reductions in *A. fumigatus* tissue burden in L-AMB, but not ABLC treated animals when compared to the 5 mg/kg/day treatment regimens (Figure 1C). The rate of fungal clearance was similar between L-AMB 10/kg/day and the ABLC 10 mg/kg/day treatment groups. Despite improvements in the rate of fungal burden reduction at the 10 mg/kg dose for L-AMB, the extent of fungal burden reduction at
the end of the experiment was similar for both formulations at the 5 and 10 mg/kg/day dosing levels.

Because early reductions in lung fungal burden appeared to correlate with a threshold concentration of AMB in the lung in the first 24 hours, fungal burden data from all treatment groups at T=24 (n=60 mice) were plotted in relation to total AMB tissue concentrations measured by HPLC (Figure 1D). A four-parameter logistic model was then fitted to the data predict threshold AMB tissue concentrations associated with growth stasis and a 1-log\textsubscript{10} reduction in \textit{A. fumigatus} CE DNA. Variability in fungal burden across all treatment groups at 24 hours could be largely explained by tissue concentrations of AMB ($R^2=0.64$). The total AMB tissue threshold concentrations associated with growth stasis and 1 log\textsubscript{10} reduction in fungal burden were 0.53 $\mu$g/g and 4.20 $\mu$g/g, respectively. At a dosage of 5 mg/kg day, 100% of ABLC treated mice vs. 20% of L-AMB treated animals achieved or surpassed tissue concentrations at 24 hours required for fungal clearance (P=0.04, two-sided Fisher exact test). At a dosage of 10 mg/kg/day, both formulations consistently (100%) achieved tissue concentrations required for fungal clearance in the first 24 hours.

**Plasma beta glucan concentrations.** Plasma beta glucan (BG) concentrations measured in infected neutropenic mice at baseline, 24, 72, and 120 hrs correlated with \textit{A. fumigatus} CE DNA fungal burden measurements determined by quantitative real-time PCR (Figure 2). Animals treated with L-AMB or ABLC at 1 mg/kg/day exhibited initial mean plasma BG concentrations of $56.9 \pm 7.1$ pg/mL that increased to $62.7 \pm 8.5$ pg/mL by 24 hours to $82.5 \pm 19.4$ pg/mL by 72 hours (Figure 1C). Similar to fungal burden data, animals treated with L-AMB 5 mg/kg/day experienced higher mean peak BG concentrations in the plasma at 24 hours versus animals treated with 5 mg/kg/day of ABLC ($84.7 \pm 8.8$ pg/mL vs. $38.16 \pm 8.1$ pg/mL, respective; P=0.017), however, plasma BG
concentrations were similar between the treatment groups at 72 and 120 hrs (Figure 1B). No difference in mean plasma BG concentrations was noted between L-AMB and ABLC treated animals at doses of 10 mg/kg/day (Figure 1C).

**Single-dose studies.** Because of the kinetic disparity in AMB delivery observed between the two formulations, we compared the duration of detectable drug concentrations in tissue and persistence of antifungal effects after single L-AMB or ABLC administered at 5 or 10 mg/kg (Figure 3). Similar to the multidose-studies, higher mean AMB lung concentrations were observed at 24 hours with ABLC vs. L-AMB 5 mg/kg single doses (20.46 μg/g vs. 3.28 μg/g P< 0.05), however tissue concentrations at subsequent timepoints were low or undetectable (< 0.25 μg/g) in both the ABLC and L-AMB treated animals (Figure 3A). A reduction in lung fungal burden was observed with ABLC treated animals at 24 and 72 hrs (Figure 1A). Similar to the multi-dose 1mg/kg/day studies, animal deaths occurred with both groups between 72-120 hours suggesting an increase in fungal burden (data not shown on graph).

In the 10 mg/kg single dose studies, tissue concentrations of AMB persisted to 72 hours but were undetectable at 120 hours (Figure 3B). Fungal clearance was equivalent between L-AMB and ABLC, but reached a plateau at 24-72 hours failing to achieve the same decrease in fungal burden reduction observed at 120 hrs in the multi-dose studies (9.44x10³ vs. 6.43x10⁴ A. fumigatus CE, respectively).

**DISCUSSION**

In this study, we compared the kinetics of AMB tissue accumulation and A. fumigatus clearance from the lung in L-AMB and ABLC -treated neutropenic mice with acute IPA. In
agreement with previous studies of experimental pulmonary aspergillosis (6, 22), we found that
treatment with L-AMB formulation was associated with effective, albeit delayed delivery of AMB in
the lung when dosed at 5 mg/kg/day that allowed fungal burden to increase in the first 24-48 hours
of treatment. In contrast, ABLC-treated animals had detectable concentrations of AMB in the lung
in the first 24 hours that surpassed MFC of the test isolate and produced evidence of fungal
clearance in the first 24-72 hours. By 120 hrs, however, no difference in fungal burden was
observed between the two formulations. Not surprisingly, these differences between these
formulations have not been confirmed in intravenous models of infection, where L-AMB is
immediately effective due to the high serum concentrations seen with intravenous dosing (10).
Moreover, when dosed at 10 mg/kg/day, both formulations achieved high concentrations in the
lung tissue in the first 24 hours and produced similar patterns of fungal clearance. The enhanced
rate of AMB distribution to the lung with 10mg/kg/day vs. 5 mg/kg/day may be a reflection of a
dose-dependent uptake of a high-dose, first-pass effect in the lung that has been reported with
other liposomal formulations (12, 13, 29).

Early clearance of A. fumigatus from the lung was associated with total AMB tissue
concentrations that surpassed the MFC of the infecting isolate. Using logistic regression, we
determined that AMB tissue concentrations of > 4 μg/g were associated with significant reductions
(1- log₁₀) in lung fungal burden as determined by quantitative real-time PCR. This threshold
concentration is in agreement with results from Olson et al., who reported that tissue
concentrations of AMB in the lungs of mice of > 3 μg/g were required for therapeutic efficacy with
L-AMB or ABLC treatment in a murine model of pulmonary aspergillosis (22). Unlike our study, the
investigators did not report any L-AMB or ABLC treatment regimens (1-12 mg/kg/day) that
achieved tissue concentrations > 10 μg/g (22). However, the investigators documented AMB
biodistribution primarily in non-infected animals, which may be diminished in the absence of hemorrhage and residual inflammatory cell recruitment that is seen in infected animals (4). Indeed, in the study by Oslon et al. tissue concentrations where disproportionately higher in infected animals treated at 15 mg/kg/day of ABLC or L-AMB (30-40 μg/g and 10-18 μg/g, respectively) compared to uninfected animals who were treated with both lipid formulations at 12 mg/kg/day (10.07 μg/g and 3.16 μg/g, respectively) (22). Fungal burden, as determined by quantitative CFU cultures, was generally lower in ABLC-treated animals with a 20 mg/kg/day regimen achieved the greatest reduction in fungal burden among all treatment groups (22).

One unexpected finding of our study was the pattern of decreasing tissue concentrations over the course of the experiment despite daily dosing. Because a majority of animal studies that have examined biodistribution of lipid AMB formulations in the lung examine only single doses or single timepoints to assess plasma: tissue ratios of drug, we are unable to confirm if this observation is indeed unique to our study (3, 6, 22). Several factors could account for decreased distribution of AMB during the course of repeated dosing in infected animals including saturation of pathways involved in drug uptake in the lung and/or decreases in residual (lipid AMB containing) phagocytic cell recruitment with decreasing fungal burden. Another possibility is that our method for AMB extraction from tissue becomes less effective as infection persists. If this were the case, we would expect a consistent pattern of decrease in all dosing regimens that was not clearly observed between the two lipid AMB formulations (Figures 1B-1C). Further studies with radiolabled AMB would be required to address this issue.

When administered as single dosages of 5 of 10 mg/kg, neither L-AMB or ABLC were as effective at reducing fungal burden as multiple daily dosing (Figure 3A and 3B). Indeed, AMB tissue concentrations were undetectable by 120 hours and fungal clearance reached a plateau.
after the first 24-48 hours. These data raise concerns whether dosing of lipid AMB formulations at extended intervals (i.e. greater than 3-5 days) would be prudent for the treatment of established IPA. However, higher-dose, infrequently-administered regimens may still be effective for lower inocula infections or in the setting of prophylaxis. Additional studies will be required to confirm the pharmacodynamics of extended interval dosing for the lipid AMB formulations.

Another novel aspect of this study is that we were able to demonstrate a good correlation between *A. fumigatus* tissue fungal burden measured by quantitative real-time PCR and serum concentrations of beta glucan (BG), including response to antifungal therapy. The BG test has been reported to be a useful adjunctive diagnostic for aspergillosis in patients with acute myelogenous leukemia, with a sensitivity of 69.9% and a specificity of 87.1% in patients with proven infections when the 60 pg/mL cutoff value is used to define sample positivity (23). However, few pre-clinical studies have compared serial measurements of BG concentrations in relation to direct measurements of fungal burden in the lungs. We decided to use beta glucan as a surrogate marker over galactomannan due to the greater dynamic range of the test; i.e. beta glucan is reported quantitatively versus the semi-quantitative galactomannan test. While the majority of the concentrations tested in this study fell below the 60 pg/mL, it is notable that samples that exceeded this threshold in animals with progressing IPA or delayed response to antifungal therapy. Additional pre-clinical and clinical studies are warranted to further explore the utility of serial BG analysis as a surrogate for response to drug therapy.

In conclusion, our comparative analysis L-AMB or ABLC in the treatment of acute experimental IPA revealed notable differences in the patterns of early AMB lung distribution and fungal clearance when the formulations were dosed at 5 mg/kg/day, but no significant differences in *A. fumigatus* clearance at 10 mg/kg/day. Differences in the speed of fungal clearance could be
largely attributed differences in the rate and extent of the respective formulations to deliver "fungicidal" concentrations of AMB to the lung tissue. While the importance of these findings remain to be determined in more slowly progressing forms of aspergillosis, our data suggest that the biopharmaceutical differences between the lipid formulations could have potentially important clinical implications for rapidly progressing fungal pneumonia (e.g., pulmonary zygomycosis). Currently, studies are underway to confirm these results with more rapidly-invasive, less AMB susceptible moulds.
ACKNOWLEDGEMENTS

Research support was provided by Enzon Pharmaceuticals.

R.E.L. and D.P.K. received research support and consultancy fees from Merck & Co., Pfizer, Astellas, Enzon, and Schering-Plough. R.A.P. receives research support from Merck & Co., Pfizer, Ortho McNeil, and Enzon. Other authors: no conflicts
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amphotericin B, itraconazole, and voriconazole against Aspergillus, Fusarium, and


FIGURE LEGENDS

Figure 1. Differences in the kinetics of AMB lung accumulation and fungal clearance between ABLC and L-AMB in a murine model of IPA. Control (○); ABLC (■); L-AMB (□). Line plots represent tissue *A. fumigatus* fungal burden expressed as conidial equivalent (CE) DNA versus time on the Y-axis. Each datum point is the mean ± standard error of five animals. Total lung AMB tissue concentrations (µg/gram) versus time are plotted on the Z-axis. Each bar represents mean tissue concentrations from five animals. A) 1 mg/kg/day dosing, B) 5 mg/kg/day dosing, C) 10 mg/kg/day dosing, and D) Relationship of 24-hour *A. fumigatus* tissue concentrations and total AMB tissue concentrations from all treatment groups (n=60 mice). Line depicts the concentration-effective relationship determined by fitting a four-parameter logistic model (Hill equation) to the data using the GraphPad Prism 4.0 software package. Mean best-fit values and 95% CI were calculated by the software. *P<0.05 of ABLC vs. L-AMB tissue concentrations determined by two-sided Mann-Whitney test.

Figure 2. Differences in concentrations of serum beta-glucan detection between ABLC and L-AMB treated mice with IPA. ABLC (■); L-AMB (□). Dotted line highlights 60 pg/mL glucan threshold considered by manufacturer to be indicative of an invasive fungal infection. A) 1 mg/kg/day dosing, B) 5 mg/kg/day dosing, C) 10 mg/kg/day dosing. *P<0.05 of ABLC vs. L-AMB determined by two-sided Mann-Whitney test.
Figure 3. Differences in the kinetics of AMB lung accumulation and fungal clearance between single-dose ABLC and L-AMB therapy in a murine model of IPA. Control (○); ABLC (■); L-AMB (□). Line plots represent tissue A. fumigatus fungal burden expressed as conidial equivalent (CE) DNA versus time on the Y-axis. Each datum point is the mean ± standard error of five animals. Total lung AMB tissue concentrations (µg/gram) versus time are plotted on the Z-axis. Each bar represents mean tissue concentrations from five animals. A) 5 mg/kg/day dosing, and B) 10 mg/kg/day dosing.
FIGURE 2

A. 1 mg/kg

B. 5 mg/kg

C. 10 mg/kg

\( \beta(1 \rightarrow 3) \) glucan pg/mL

Time (hr)

FIGURE 3

A. H. capsulatum CE (qPCR)

B. A. fumigatus CE (qPCR)

Time (hr)

* P < 0.05